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New Hepatitis E Virus Genotype in Bactrian Camels, Xinjiang, China, 2013

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To the Editor: Hepatitis E virus (HEV) is a member of the family *Hepeviridae*, genus *Orthohepevirus*, which comprises 4 species, *Orthohepevirus A–D. Orthohepevirus A* contains 7 genotypes (HEV1–7) (*1,2*). HEV1 and HEV2 infect humans only; HEV3, HEV4, and HEV7 can infect humans and other mammals; and HEV5 and HEV6 have been detected in animals only.

Worldwide, HEV is the most common cause of acute viral hepatitis in humans. The disease is generally self-limiting, but high death rates have been observed among HEV-infected pregnant women. Chronic HEV infection is a problem in immunocompromised patients, such as solid organ transplant recipients (*3*). Human HEV3 and HEV4 infections have been associated with consumption of undercooked pork or game meat *(4)*.

In 2014, we described the discovery of a novel genotype of HEV in dromedaries (*Camelus dromedarius* or 1-humped camels), suggesting another possible source of human HEV infection (*5*). This dromedary HEV was subsequently classified as a novel *Orthohepevirus A* genotype,
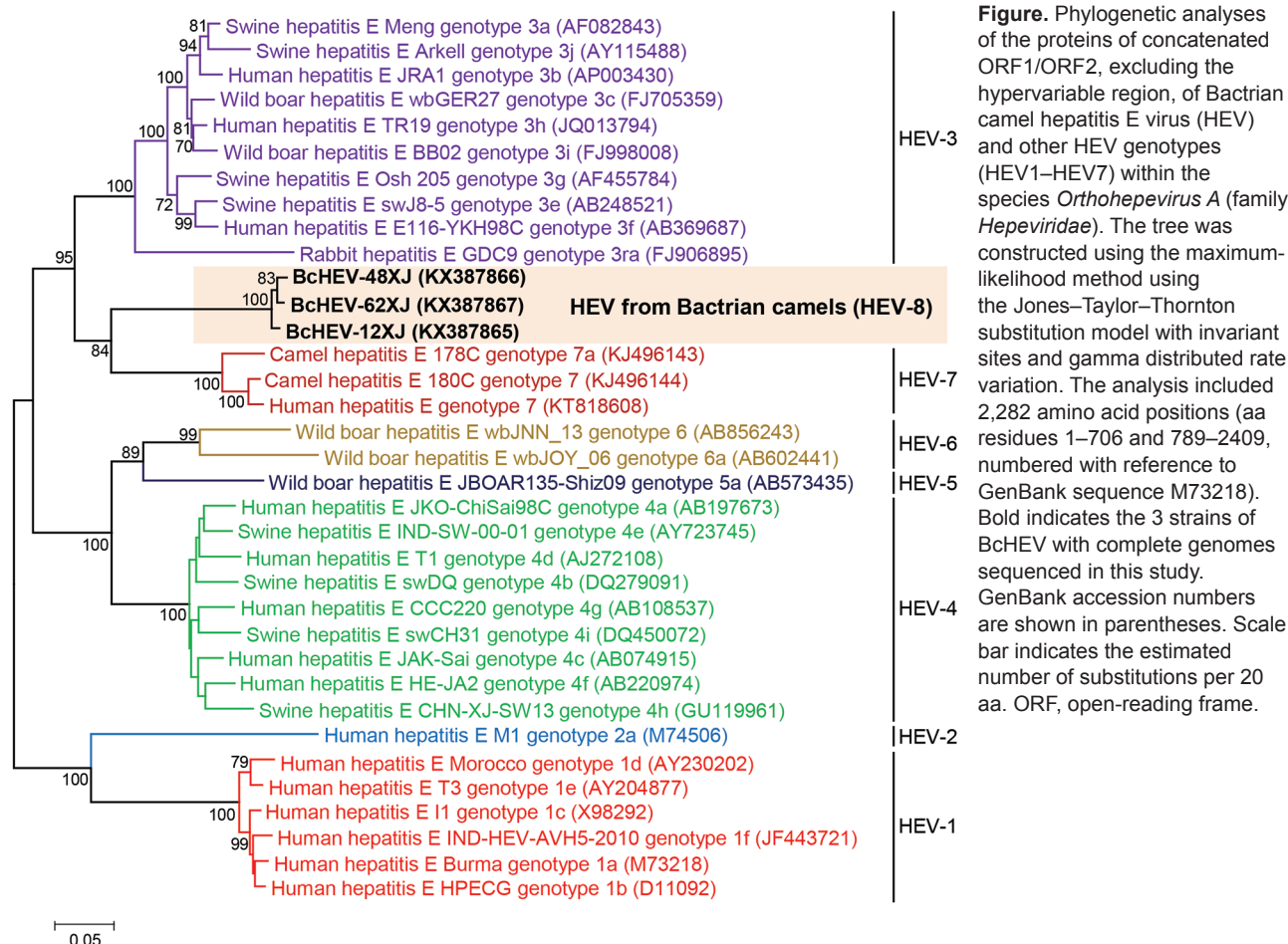
HEV7 (*1,2*). Recently, this HEV7 genotype was also isolated from a liver transplant recipient from the Middle East with chronic HEV infection (*6*). The patient regularly consumed dromedary camel meat and milk, implying camel-to-human transmission of the virus (*6*).

Like the dromedary, the Bactrian camel (*Camelus bactrianus* or 2-humped camels) is an Old World camelid species. Thus, we hypothesize that Bactrian camels may also be reservoirs of HEV. To test this hypothesis and increase our understanding of the epidemiology of HEV in camels, we performed a molecular epidemiology study using feces samples from camels in China.

During November 2012–May 2013, we collected and tested 1 feces sample each from 205 Bactrian camels on a farm in Xinjiang, China. We performed RNA extraction and reverse transcription PCR (RT-PCR) as previously described (*7*). We screened for HEV by PCR amplification of a 251-bp fragment of open-reading frame (ORF) 2, using primers 5′-GTTGTCTCAGCCAATGGCGA-3′ and 5′-GTAGTTTGGTCATACTCAGCAGC-3′. PCR was performed, using previously described conditions (*7*), with the annealing temperature set at 50°C. DNA sequencing and quantitative real-time RT-PCR were performed as previously described (*7*). Three samples were positive for HEV; we performed complete genome sequencing of these samples as described (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/12/16-0979-Techapp1.pdf) (*5,7*). We also performed comparative genomic analysis as previously described (*1,2,8*). We constructed a phylogenetic tree using the maximum-likelihood method and MEGA7 (*9*); bootstrap values were calculated from 1,000 trees. The optimal substitution model for each ORF was selected by MEGA7 (Figure).

RT-PCR for a 251-bp fragment in ORF2 of HEV was positive for 3 (1.5%) of the 205 fecal samples; virus loads were 1.6×10^3, 2.1×10^3, and 1.8×10^4 copies/mg, respectively. Whole-genome sequencing of the 3 Bactrian camel HEV (BcHEV) strains (GenBank accession nos. KX387865–7) showed genome sizes of 7,212–7,223 bp and a G + C content of 52.7%–53.1%. Overall, nucleotides in the BcHEV genome differed by >20% compared with those in all other HEVs. Genomes of the 3 BcHEV isolates contained 3 major ORFs; genome organization was typical of and characteristics were similar to those of HEVs from other *Orthohepevirus A* species. Phylogenetic trees constructed using ORF1, ORF2, ORF3, and concatenated ORF1/ORF2, excluding the hypervariable region, showed that these 3 BcHEV isolates clustered with the 2 dromedary camel HEV7 strains and the HEV7 strain from the liver-transplant recipient with chronic hepatitis (Figure; online Technical Appendix Figure 1)

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[1]These authors contributed equally to this article.

Figure. Phylogenetic analyses of the proteins of concatenated ORF1/ORF2, excluding the hypervariable region, of Bactrian camel hepatitis E virus (HEV) and other HEV genotypes (HEV1–HEV7) within the species *Orthohepevirus A* (family *Hepeviridae*). The tree was constructed using the maximum-likelihood method using the Jones–Taylor–Thornton substitution model with invariant sites and gamma distributed rate variation. The analysis included 2,282 amino acid positions (aa residues 1–706 and 789–2409, numbered with reference to GenBank sequence M73218). Bold indicates the 3 strains of BcHEV with complete genomes sequenced in this study. GenBank accession numbers are shown in parentheses. Scale bar indicates the estimated number of substitutions per 20 aa. ORF, open-reading frame.

(*5,6*). However, amino acid distances based on the concatenated ORF1/ORF2, excluding the hypervariable region of the 3 BcHEV isolates and the existing genotypes, ranged from 0.095 to 0.148, which was greater than the threshold (p-distance = 0.088) to demarcate intergenotype distance (*1,2*). Using this criterion, we propose that the 3 BcHEV isolates should constitute a new HEV genotype, HEV8.

A recent study in Dubai, United Arab Emirates, showed that HEV accounted for 40% of acute hepatitis cases (*10*). Even though HEV is an emerging pathogen in the Middle East, limited sequence data exist regarding the virus on the Arabian Peninsula. Recently, we discovered the HEV7 genotype in 1.5% of 203 feces samples from dromedaries in Dubai (*5*). In the current study, we detected a new HEV genotype in 1.5% of 205 Bactrian camels on a farm in Xinjiang. Comparative genomic and phylogenetic analyses showed that BcHEV represents a previously unrecognized HEV genotype. It has been shown that HEV7 from dromedaries can be transmitted to humans; thus, meat and milk from Bactrian camels might pose a similar risk to humans. The increasing discoveries of camel viruses and of their transmission to humans highlight the need for caution when handling these mammals and processing food products derived from them.

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Avian Influenza Virus H5 Strain with North American and Eurasian Lineage Genes in an Antarctic Penguin

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To the Editor: Previous studies have reported avian influenza virus (AIV)–positive serum samples obtained from Adélie (*Pygoscelis adeliae*), chinstrap (*Pygoscelis antarcticus*), and gentoo (*Pygoscelis papua*) penguins (*1–4*). Only recently was an H11N2 subtype virus isolated from Adélie penguins in Antarctica (*5*). We performed AIV surveillance in the Antarctic Peninsula to identify the strains currently circulating in different penguins species in this area.

During 2015–2016, we sampled penguin colonies from 9 locations on the Antarctic Peninsula. We collected 138 blood samples from Adélie penguins at Ardley Island (62°13′S, 58°56′W), Arctowski Base (62°9′S, 58°28′W), and Bernardo O'Higgins Base (63°19′S, 57°53′W) and identified 5 serum samples positive for influenza. We also collected 513 cloacal swabs from Adélie, chinstrap (online Technical Appendix Figure 1, panel A; http://wwwnc.cdc.gov/EID/article/22/12/16-1076-Techapp1.pdf), and gentoo penguins from Mikkelsen Harbor (63°54′S, 60°47′W), Dorian Bay and Port Lockroy (64°48′S, 63°30′W), Pleneau Island (65°06′S, 64°04′W), Brown Base (64°53′S, 62°52′W), Orne Harbor (64°37′S, 62°32′W), and Aitcho Island (62°23′S, 59°46′W) during January–March of 2 consecutive seasons (2015 and 2016; online Technical Appendix Figure 1, panel B; http://wwwnc.cdc.gov/EID/article/22/12/16-1076-Techapp1.pdf). Quantitative reverse transcription PCR (RT-PCR) analysis of the matrix segment (*6*) identified 21 positive AIV samples from penguins (8 chinstrap, 13 gentoo) on Aitcho Island, demonstrating the presence of AIV in 2 additional penguin species in a new location in Antarctica.

Using multisegment RT-PCR performed with influenza-specific universal primers, we amplified all 8 virus segments from a chinstrap penguin specimen, which yielded cDNA products suitable for next-generation sequencing with a HiSeq 2500 System (Illumina, San Diego, CA, USA). This virus was subtyped as an H5N5 and named A/chinstrap_penguin/Antarctica/B04/2015 (H5N5). Analysis of its cleavage site confirmed this was a typical low pathogenicity AIV (LPAIV) containing cleavage motif PQRETRGLF (*7*).

To trace the origin of this H5N5 virus, we performed phylogenetic analyses of its hemagglutinin and neuraminidase genes (Figure, panels A, B; online Technical Appendix Figures 2, 3, http://wwwnc.cdc.gov/EID/article/22/12/16-1076-Techapp1.pdf). The hemagglutinin gene was placed

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Technical Appendix

Complete Genome Sequencing

Three complete genomes of Bactrian camel HEV (BcHEV) strains, including BcHEV-12XJ, BcHEV-48XJ and BcHEV-62XJ, were amplified and sequenced using the RNA extracted from the original specimens as templates. The RNA was converted to cDNA by a combined random-priming and oligo(dT) priming strategy. The cDNA was amplified by primers designed by multiple alignments of the genomes of other HEVs with complete genomes available. Additional primers were designed from the results of the first and subsequent rounds of sequencing (Table). The 5′ ends of the viral genomes were confirmed by rapid amplification of cDNA ends using the 5′/3′ RACE kit (Roche, Germany). Sequences were assembled and manually edited to produce final sequences of the viral genomes.

Technical Appendix Table. Primers for amplification of the three BcHEV genomes

Primers	5′ to 3′
Forward	
LPW28520	GTTGTCTCAGCCAATGGCGA
LPW28892	CGAAGGCTTACGAATGTTGC
LPW29061	ATCCGTTGGTCATTGAGA
LPW29066	ACTGTTGAGCTTACAGTTG
LPW29070	TGCATGGTGTTTGAGAATGA
LPW31223	CCGGCCCCTACAGTCTTTCATAT
LPW31225	CCGCTAATCCTGGTGCTATTA
LPW29072	TGCTGGACTTGACTAACTCA
LPW31228	GTATTGCCTCTGAACTTGT
LPW31226	GTACGAAGCTGTATGAAGCTGCTCA
LPW31432	GAAGGGTCTGAGGTCGATT
LPW31299	GGCTGTACTGTTGCTGTTCCT
LPW32175	CCATGTGTGGGAGTCCAA
Reverse	
LPW28521	GTAGTTTGGTCATACTCAGCAGC
LPW28893	CTGAGAATCAACCCGGTCA
LPW29062	CAACTGTAAGCTCAACAGT
LPW29067	CGAGTGAGTGCAACAATAGCA
LPW29071	GCTGAGAATCAACCCGGTCA
LPW31231	CTTACCAGAACCAGGGACA
LPW31233	CAGAACCCTTTCAGAGACTCCTT
LPW28521	GTAGTTTGGTCATACTCAGCAGC
LPW29077	GCCCTGAGTGTAATTCTCTT
LPW31233	CAGAACCCTTTCAGAGACTCCTT

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LPW31433        GCATGTGCACGAGAAGATT
LPW32181        GCATAATTGGACGCCTCAG
LPW31433        GCATGTGCACGAGAAGATT
```

A

- Human hepatitis E JRA1 genotype 3b (AP003430)
- Swine hepatitis E Arkell genotype 3j (AY115488)
- Swine hepatitis E Meng genotype 3a (AF082843)
- Human hepatitis E TR19 genotype 3h (JQ013794)
- Wild boar hepatitis E BB02 genotype 3i (FJ998008)
- Wild boar hepatitis E wbGER27 genotype 3c (FJ705359)
- Swine hepatitis E Osh 205 genotype 3g (AF455784)
- Swine hepatitis E swJ8-5 genotype 3e (AB248521)
- Human hepatitis E E116-YKH98C genotype 3f (AB369687)
- Rabbit hepatitis E GDC9 genotype 3ra (FJ906895)

HEV-3

- BcHEV-48XJ (KX387866)
- BcHEV-62XJ (KX387867)
- BcHEV-12XJ (KX387865)

HEV from Bactrian camels (HEV-8)

- Camel hepatitis E 178C genotype 7a (KJ496143)
- Camel hepatitis E 180C genotype 7 (KJ496144)
- Human hepatitis E genotype 7 (KT818608)

HEV-7

- Wild boar hepatitis E wbJNN_13 genotype 6 (AB856243)
- Wild boar hepatitis E wbJOY_06 genotype 6a (AB602441)

HEV-6

- Wild boar hepatitis E JBOAR135-Shiz09 genotype 5a (AB573435)

HEV-5

- Human hepatitis E CCC220 genotype 4g (AB108537)
- Swine hepatitis E swCH31 genotype 4i (DQ450072)
- Human hepatitis E JAK-Sai genotype 4c (AB074915)
- Human hepatitis E HE-JA2 genotype 4f (AB220974)
- Swine hepatitis E CHN-XJ-SW13 genotype 4h (GU119961)
- Swine hepatitis E swDQ genotype 4b (DQ279091)
- Human hepatitis E JKO-ChiSai98C genotype 4a (AB197673)
- Human hepatitis E T1 genotype 4d (AJ272108)
- Swine hepatitis E IND-SW-00-01 genotype 4e (AY723745)

HEV-4

- Human hepatitis E M1 genotype 2a (M74506)

HEV-2

- Human hepatitis E Morocco genotype 1d (AY230202)
- Human hepatitis E T3 genotype 1e (AY204877)
- Human hepatitis E I1 genotype 1c (X98292)
- Human hepatitis E IND-HEV-AVH5-2010 genotype 1f (JF443721)
- Human hepatitis E Burma genotype 1a (M73218)
- Human hepatitis E HPECG genotype 1b (D11092)

HEV-1

0.05

B

- Human hepatitis E Burma genotype 1a (M73218)
- Human hepatitis E Morocco genotype 1d (AY230202)
- Human hepatitis E IND-HEV-AVH5-2010 genotype 1f (JF443721)
- Human hepatitis E HPECG genotype 1b (D11092)
- Human hepatitis E T3 genotype 1e (AY204877)
- Human hepatitis E I1 genotype 1c (X98292)

HEV-1

- Human hepatitis E M1 genotype 2a (M74506)
- Human hepatitis E Nig7 genotype 2b (AF173231)

HEV-2

- BcHEV-12XJ (KX387865)
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HEV-7

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- Swine hepatitis E Arkell genotype 3j (AY115488)
- Human hepatitis E JRA1 genotype 3b (AP003430)
- Rabbit hepatitis E GDC9 genotype 3ra (FJ906895)
- Wild boar hepatitis E BB02 genotype 3i (FJ998008)
- Human hepatitis E TR19 genotype 3h (JQ013794)
- Wild boar hepatitis E wbGER27 genotype 3c (FJ705359)
- Human hepatitis E E116-YKH98C genotype 3f (AB369687)
- Swine hepatitis E swJ8-5 genotype 3e (AB248521)
- Swine hepatitis E Osh 205 genotype 3g (AF455784)

HEV-3

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HEV-6

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- Swine hepatitis E CHN-XJ-SW13 genotype 4h (GU119961)
- Human hepatitis E CCC220 genotype 4g (AB108537)
- Swine hepatitis E swCH31 genotype 4i (DQ450072)

HEV-4

0.02

C

- Human hepatitis E JAK-Sai genotype 4c (AB074915)
- Swine hepatitis E CHN-XJ-SW13 genotype 4h (GU119961)
- Swine hepatitis E swDQ genotype 4b (DQ279091)
- Human hepatitis E T1 genotype 4d (AJ272108)
- Swine hepatitis E IND-SW-00-01 genotype 4e (AY723745)
- Human hepatitis E CCC220 genotype 4g (AB108537)
- Swine hepatitis E swCH31 genotype 4i (DQ450072)
- Human hepatitis E HE-JA2 genotype 4f (AB220974)
- Human hepatitis E JKO-ChiSai98C genotype 4a (AB197673)

HEV-4

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HEV-5

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- Wild boar hepatitis E wbJOY_06 genotype 6a (AB602441)

HEV-6

- Rabbit hepatitis E GDC9 genotype 3ra (FJ906895)
- Human hepatitis E TR19 genotype 3h (JQ013794)
- Swine hepatitis E Meng genotype 3a (AF082843)
- Human hepatitis E JRA1 genotype 3b (AP003430)
- Wild boar hepatitis E BB02 genotype 3i (FJ998008)
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- Wild boar hepatitis E wbGER27 genotype 3c (FJ705359)
- Swine hepatitis E Osh 205 genotype 3g (AF455784)
- Swine hepatitis E swJ8-5 genotype 3e (AB248521)
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- Human hepatitis E T3 genotype 1e (AY204877)
- Human hepatitis E Morocco genotype 1d (AY230202)
- Human hepatitis E Burma genotype 1a (M73218)
- Human hepatitis E HPECG genotype 1b (D11092)

HEV-1

0.05

Technical Appendix Figure. Phylogenetic analyses of A) ORF1, B) ORF2, and C) ORF3 and other genotypes of HEVs (HEV-1 to HEV-7) within the species *Orthohepevirus A*. The trees were constructed using maximum likelihood method and the optimal substitution models of JTT+G+I+F, JTT+G+I and JTT+G were used for ORF1, ORF2 and ORF3, respectively. Amino acid residues 1–1743, 1–660 and 10–123 in ORF1, ORF2 and ORF3, numbered with reference to GenBank sequence M73218, were included in the analyses. For ORF1 and ORF3, the scale bars indicate the estimated number of substitutions per 20 aa. For ORF2, the scale bar indicates the estimated number of substitutions per 50 aa. The three strains of DcHEV with complete genomes sequenced in this study are in bold.